IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: John J. Harrington *et al.* Serial No.: 09/484,331 Filed: January 18, 2000 For: COMPOSITIONS AND METHODS FOR NON-TARGETED ACTIVATION OF ENDOGENOUS GENES Attorney Docket No.: ATX-007CP4DV12 (formerly 5817-L)	Group Art Unit: 1655 Examiner: Shukla, Ram R.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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10 July 2003
Date of Signature and of Mail Deposit

By: Lisa L. Pringle
Lisa L. Pringle

REVOCATION OF PRIOR POWERS OF ATTORNEY AND APPOINTMENT OF NEW POWER OF ATTORNEY

Dear Sir:

Athersys, Inc., the Assignee of the entire right, title and interest in the above-identified application by virtue of the Assignment document recorded in the U.S. Patent and Trademark Office for U.S. Serial No. 09/760,897 on 2 July 1999 (reel 010064, frame 0420) hereby revokes all Powers of Attorney with respect to that application heretofore granted and appoints the below listed attorneys with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office connected therewith.

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Title: Executive Vice President and COO

Dated: 7/3/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Application of

Harrington J. *et al.*

Art Unit: 1632

Application No.: 09/484,331

Examiner: R. Shukla

Filed: January 18, 2000

Atty. Docket: 0221-0003L

For: Compositions and Methods
For Non-Targeted Activation
of Endogenous Genes

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner of
Patents and Trademark
Washington, D.C. 20231

Sir:

The undersigned, Dale S. Dhanoa, Ph.D., declares and states:

I am currently Senior Vice President of Research and Discovery at Predix Pharmaceuticals, Inc. in Woburn, Massachusetts. I submit that I specialize in the fields of drug discovery, design, synthesis, biological and pharmacological characterization of molecules (both non-peptide and peptide) for drug development, high throughput screening, binding assays, functional assays, cell-based assays, medicinal chemistry, combinatorial chemistry, and computational chemistry, as evidenced by my attached resume and the publication list. See, in particular, the short list of references 1-20 attached to this Declaration. Based on my knowledge and experience, I believe I am

qualified to speak to the skill and understanding of the person of ordinary skill in the art of drug discovery.

I have read U.S. Patent Application No. 09/941,223, filed September 26, 1997, in its entirety. I have noted the sections that specifically mention the drug discovery process. These include page 5, last paragraph; page 9, fourth full paragraph; page 12, last paragraph; page 16, first and second full paragraphs; and page 45, third full paragraph. However, I have read the entire application to put the reference to drug discovery in the context of the entire technology in the application. I have also read claims 62-69 (see copy attached).

From the entire application, I understand the technology as introducing a vector into the genome of a cell by random integration and activating one or more genes in that cell by means of a transcriptional regulatory sequence, such as a promoter, on the vector. This means that the methods can be used to randomly activate genes and cells can be produced that express a gene of interest or acquire a phenotype of interest from activation by the vector. I refer to the technology as the "RAGE" technology. To me this covers methods of activation and products of activation, such as RAGE-activated cells and gene products from RAGE-activated cells.

With respect to the time period in which my statements apply, my frame of reference would be, at the earliest, the time of September 26, 1997. This is the time that the patent application that I have reviewed was filed. I have refreshed my memory as to

my understanding of the state of art of drug discovery on and about that time by recalling the projects I worked on, the scientific literature and conferences, my publications and presentations, as well as my personal notes and mementos from that time period. See my resume, attached.

The issue, as I understand it, is that the Patent Examiner believes that the application fails to convey, to the person of ordinary skill in the field of drug discovery, the step of screening a test compound on a RAGE-activated cell.

I do not agree with this position for the reasons that follow.

Drug Discovery: Summary Introduction

The drug discovery process, at a minimum, involved screening a test compound for a desired effect on a gene product or on a phenotype.

It was understood that drug discovery needed an end point to assess qualitatively or quantitatively the effect of a compound or compounds on the activation or inactivation (stimulation or agonist activity measurement or inhibition or antagonist activity measurement, respectively) of functional activity of a protein in a cell. Typically, two approaches were most commonly used: protein-based screening (included cell lysate, membrane-bound or fully/partially purified protein) and cell-based screening.

Generally, protein-based screening was utilized for determining the binding affinity of compounds against the target protein (for example, G protein-coupled receptors) or inhibitory potency (percent inhibition) against soluble protein (for example, HIV protease).

Protein-based assay measures the binding affinity of compounds with the protein but not its functional activity, meaning whether it will block or activate the biological function (response) of the target protein. These *in vitro* assays alone, however, would not demonstrate functional activity in cells expressing target protein or directly test its efficacy in animals.

Hence, besides determining the *in vitro* activities, these compounds would have been tested in whole cell assays. Whole cell assays were actually a better measure of the performance of test compounds if one was testing for the effect of the compound on a cellular process. In fact, screening compounds directly in cell-based assays was a more efficient process.

Cell-based assays can directly give the compound's functional activity against a protein or proteins. This approach is one step closer to the target and can bypass the protein isolation/purification. Cell-based assays will give somewhat qualitative measure of a compound's biological activity.

Drug Discovery: Detailed Explanation

Any potential drug must demonstrate efficacy in cells, tissues, or animals in its preclinical research and ultimately in humans. During the lead discovery and optimization of drug development candidates, knowing the structure-activity data of drug candidates is extremely essential. This is acquired by a number of ways: protein-ligand binding affinity and/or biological activity measured by cell-based assays.

These data may be generated by screening compounds against a target by using the protein and/or cell-based assays. Even if the affinity of a particular compound (hit/lead/drug) is already determined against a target protein, it is then tested in cells (or tissues) expressing the target protein to determine its functional activity in a biological system. This screen could also be used as a primary screen as well to identify initial active compounds (aka hit/lead). The cell-based approach is one of the most important methods available over the last several decades that has been driving drug discovery forward by providing this invaluable information for potential drug property optimization including its potency, specificity, functional activity, toxicity and cell-penetration.

Cell-based assay is a term used to refer to a number of different experiments using living cells. The general definition can include a variety of assays that measure cell proliferation, functional activity, toxicity, and motility. Cell-based assays differ from screening against proteins (enzymes, receptors, kinases) or antibody-based assays.

Cell-based assays provided a more accurate representation of the real-life model since living cells are used.

Cell functions are an aggregate of many interacting signaling and feedback biological pathways. Compound screening using isolated protein targets cannot consider this complexity. Thus, testing of compounds (drug leads and/or drug candidates and/or drugs) in whole cells provided a much more complete understanding of the effects of the compounds as potential drugs. The advantages of employing cell based assays for compound screening included:

- Efficacy determination by measuring function and biological behavior in cells
- Evaluation of molecular interactions within the inside environment of the cells
- Evaluation of drug penetration at early stages in whole cells
- Evaluation of compound toxicity and non-specific effects on cells
- Identification of orphan targets whose function and identity is unknown require cell-based functional assays
- No protein purification and isolation required
- Powerful in identifying false-positives earlier in drug discovery, unlike protein-based assays

Cell-based assays were used in addition to (not in lieu of) protein-based screening assays for compound screening and it was a necessity for screening selected (or all) compounds to eliminate false-positives and identify potential drug candidates. *It was not a matter of either protein-based or cell-based assays, but instead both these approaches were routinely used in drug discovery.* Moreover, cell-based assays were more essential

than protein-based assays for drug screening and drug-profiling as cell-based screens were better representatives of the *in vivo* biological systems to evaluate fate of compounds as drugs.

Selected examples out of several successful drug discovery programs, carried out on or before September 26, 1997, where both cell-based assays and protein-based assays have been used include:

- Angiotensin II receptor antagonist drug, Cozaar (Losartan) for treating hypertension
- Endothelin receptor antagonist drug, Tracleer (Bosentan) for treating acute pulmonary hypertension
- HIV Protease inhibitor drug, Crixivan, for AIDS
- Neurokinin receptor antagonist drug, Emend, for treating emesis
- Corticotrophin Releasing Hormone Receptor antagonists for the potential treatment of stress related diseases, including anxiety and depression etc.

Protein-based screening identified binders (ligands/compounds) and needed cell-based assays, as well as animal studies, to identify potential drugs, while cell-based assays identified entities that are not only biologically active in *ex-vivo* testing, but have a much greater chance of being active in *in vivo* also.

The Patent Application

The application in several places, as I indicated above, refers to the use of the RAGE technology in drug discovery. Because the state of the art of drug discovery, as

described above, at the application's filing date of September 26, 1997, the cited passages, within the full context of the application, would have indicated to me that a compound could be tested against a RAGE cell with an activated gene of interest to determine if the compound affects the gene of interest or phenotype of interest.

The patent application refers to using the purified protein for drug discovery. (See the application page 5, last paragraph; page 16, lines 12-13; page 12, last paragraph.) As stated above, this was typically done when the test was for a compound that binds to the protein. However, this was not a necessary step. Often, especially in preliminary drug discovery, it was undesirable to go through the steps of actually purifying a specific protein. Therefore, compounds would have been tested in cellular lysates or against whole cells. This would have been a highly preferred way to do initial compound testing. In fact, for assessing the effect on a cellular process, a whole cell assay was the only way.

The application also indicates that cells can be cultured *in vitro* and used for drug discovery. (See the application, page 9, lines 12-15; page 45, lines 22-25.) This means to me that the RAGE cell with the activated gene is exposed to a test compound and then the effect of the compound is assessed.

I also believe that a person of ordinary skill in the field, having read the application, would have realized that what is intended by the application is to expose a test compound to a gene product both by way of a protein product *in vitro* (as explicitly stated) and also by way of a whole cell assay (as clearly implied).

Based on my reading of the patent application, therefore, it is my opinion that the person of ordinary skill in the field of drug discovery, reading this application on or about the filing date of September 26, 1997, would have realized that the Applicants, by mentioning the drug discovery process as they did, implicitly were describing the drug discovery method in claims 62-69, copy attached.

Dale S. Dhanoa

Dale S. Dhanoa, Ph.D.

July 10, 2003

Date

Attachment for Declaration

62. (Once amended) A method for drug discovery comprising:

(a) integrating a vector into the genome of one or more eukaryotic cells, wherein said vector integration activates expression of an endogenous gene in said one or more cells;

(b) culturing said one or more cells under conditions favoring expression of said activated gene, thereby producing a gene product of said activated gene;

(c) screening said one or more cells for a cell in which a desired gene is activated or for a cell in which a desired phenotype is induced by said activated gene;

(d) treating said cell, in which said desired gene is activated or in which said desired phenotype is induced, with one or more test compounds to be screened for drug activity; and

(e) determining the ability of said one or more test compounds to interact with a product of said desired activated gene.

63. A method for drug discovery comprising:

(a) integrating a vector into the genome of one or more eukaryotic cells, wherein said vector integration activates expression of an endogenous gene in said one or more cells;

(b) culturing said one or more cells in reduced-serum cell culture medium under conditions favoring production of a protein encoded by said activated gene and secretion of said protein into the cell culture medium;

(c) screening said one or more cells for a cell in which a desired gene is activated and the protein encoded by said desired gene is secreted into the cell culture medium; and

(d) screening one or more test compounds for drug activity by determining the ability of said test compounds to interact with said secreted protein in said cell culture medium.

64. The method of claim 63, further comprising concentrating said cell culture medium prior to said screening in (d).

65. The method of claim 63, further comprising isolating said protein prior to said screening in (d).

66. The method of claim 62 wherein said vector comprises a transcriptional regulatory sequence and wherein expression of said endogenous gene is activated by means of said transcriptional regulatory sequence.

67. The method of claim 63 wherein said vector comprises a transcriptional regulatory sequence and wherein expression of said endogenous gene is activated by means of said transcriptional regulatory sequence.

68. The method of any of claims 62–67 wherein said vector integrates into the genome by non-homologous recombination.

69. (New) A method for drug discovery comprising:

(a) integrating a vector, comprising a promoter, into the genome of one or more eukaryotic cells, by non-homologous recombination, wherein said promoter activates expression of an endogenous gene in said one or more cells;

(b) culturing said one or more cells under conditions favoring expression of said activated gene, thereby producing a gene product of said activated gene;

(c) screening said one or more cells for a cell in which a desired gene is activated or for a cell in which a desired phenotype is induced by said activated gene;

(d) treating said cell, in which said desired gene is activated or in which said desired phenotype is induced, with one or more test compounds to be screened for drug activity; and

(e) determining the ability of said one or more test compounds to interact with a product of said desired activated gene or to affect said desired phenotype.